

Whole-Leaf Wash Improves Chlorine Efficacy for Microbial Reduction and Prevents Pathogen Cross-Contamination during Fresh-Cut Lettuce Processing

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Abstract: Currently, most fresh-cut processing facilities in the United States use chlorinated water or other sanitizer solutions for microbial reduction after lettuce is cut. Freshly cut lettuce releases significant amounts of organic matter that negatively impacts the effectiveness of chlorine or other sanitizers for microbial reduction. The objective of this study is to evaluate whether a sanitizer wash before cutting improves microbial reduction efficacy compared to a traditional postcutting sanitizer wash. Romaine lettuce leaves were quantitatively inoculated with *E. coli* O157:H7 strains and washed in chlorinated water before or after cutting, and *E. coli* O157:H7 cells that survived the washing process were enumerated to determine the effectiveness of microbial reduction for the 2 cutting and washing sequences. Whole-leaf washing in chlorinated water improved pathogen reduction by approximately 1 log unit over traditional cut-leaf sanitization. Similar improvement in the reduction of background microflora was also observed. Inoculated “Lollo Rossa” red lettuce leaves were mixed with noninoculated Green-Leaf lettuce leaves to evaluate pathogen cross-contamination during processing. High level (96.7% subsamples, average MPN 0.6 log CFU/g) of cross-contamination of noninoculated green leaves by inoculated red leaves was observed when mixed lettuce leaves were cut prior to washing in chlorinated water. In contrast, cross-contamination of noninoculated green leaves was significantly reduced (3.3% of subsamples, average MPN ≤ -0.3 log CFU/g) when the mixed leaves were washed in chlorinated water before cutting. This result suggests that whole-leaf sanitizing washes could be a practical strategy for enhancing the efficacy of chlorine washes for pathogen reduction and cross-contamination prevention.

Keywords: chlorine, *E. coli* O157:H7, lettuce, microbial reduction, whole-leaf wash

Practical Application: Freshly cut leafy greens release large amount of organic matter that negatively impact the chlorine washing efficacy. Implementing the primary antimicrobial intervention step of chlorine washing prior to cutting can significantly improve the efficacy of microbial reduction and minimize pathogen cross-contamination.

Introduction

Fresh and fresh-cut leafy green vegetables are nutrient-rich foods with high levels of minerals, vitamins, and phytochemicals. However, recent outbreaks of food-borne illness associated with fresh produce have negatively impacted consumer confidence in the safety of fresh and fresh-cut produce. Between 1996 and 2006, more than 20 food-borne illness outbreaks were traced back to fresh and fresh-cut leafy green vegetables, and the Center for Disease Control and Prevention (CDC) considers leafy green vegetables as one of the most important vehicles of food-borne illness

outbreaks caused by bacterial pathogens including *Escherichia coli* O157:H7 (Herman and others 2008; Lynch and others 2009).

Leafy green vegetables grow in the natural environment, and can be contaminated by bacterial pathogens such as *E. coli* O157 and *Salmonella* during growth, harvesting, and transportation (Wachtel and others 2002; Solomon and others 2003; Islam and others 2004; Jay and others 2007; Franz and van Bruggen 2008; Kim and Harrison 2008; Taormina and others 2009). Good Agricultural Practices (GAP) are increasingly emphasized (FDA 2006; CALGMA 2007) to minimize produce exposure to sources of bacterial pathogens, including contaminated water, soil, wild and domestic animals. However, occasional inadvertent contamination of leafy greens on farms by bacterial pathogens can still occur, leading to contaminated produce entering the processing lines for fresh-cut products in processing plants. It is imperative that the fresh-cut processing effectively reduces the initial contamination to ensure the safety of fresh-cut produce.

Chlorine is the most widely used sanitizer in leafy green washing because of its low cost and proven efficacy against bacterial pathogens (Adams and others 1989; Beuchat 2004; Gil and others 2009). Proper application of chlorine washing can reduce

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microbial loads on leafy green produce and, more importantly, prevents initially localized contamination from spreading during processing. However, like other oxidant sanitizers, chlorine efficacy is affected by the organic loads in the washing solution (Gonzalez and others 2004). Freshly cut lettuce can release copious amounts of vegetable latex into the washing solution and rapidly deplete free chlorine, leading to diminished efficacy against potential pathogen contaminants. Therefore, controlling the organic matter entering into the washing system is an effective means of enhancing and maintaining chlorine wash efficacy.

Although it is well recognized that organic matter from freshly cut lettuce negatively impacts the efficacy of sanitizer washing, at present nearly all fresh-cut leafy green processing facilities in the United States are configured to wash lettuce in chlorine or other sanitizer solutions immediately after cutting. The objective of this study is to evaluate the potential benefits in terms of pathogen reduction efficacy of altering the current cutting then washing sequence so that lettuce is cut only after sanitizer (chlorine) washing. We present experimental data here to demonstrate that a chlorinated water wash before cutting can significantly improve pathogen reduction efficacy in comparison to a chlorine wash after cutting. Moreover, data show that sanitizing wash in chlorinated water prior to cutting effectively prevented pathogen cross-contamination during leafy green processing.

Materials and Methods

Plant materials

Romaine, Green-Leaf, and Lollo Rossa lettuce (*Lactuca sativa* L.) were used in the studies. The lettuce heads were purchased from a produce wholesale market (Jessup, Md., U.S.A.) and stored at 5 °C overnight, before use the following day. The lettuce heads were trimmed to remove old and damaged leaves and cored before inoculation and other experimental purposes. Romaine lettuce was used in the pathogen reduction studies, while Lollo Rossa and Green-Leaf lettuce were selected for the cross-contamination study to take advantage of their color contrast.

Bacterial strains

Nonpathogenic *E. coli* O157:H7 strains CDC B6914/pGFP (ampicillin resistant), ATCC 43888, and ATCC 700728 were used in this study. A spontaneous nalidixic acid resistant mutant of ATCC 43888 and a spontaneous rifampicin resistant mutant of ATCC 700728 were selected by heavily streaking the parental strains on CT (cefexime, 0.05 mg/L and potassium tellurite, 2.5 mg/L. Invitrogen, Carlsbad, Calif. U.S.A.) supplemented sorbital MacConkey (Neugen, Lansing, Mich., U.S.A) (CT-SMAC) plates containing a gradient (0 to 100 mg/L) of appropriate antibiotics. The stability of the antibiotic markers was confirmed by quantitative comparison of growth on CT-SMAC plates with and without the antibiotics following repeated subculturing without the antibiotics in tryptic soy broth (TSB, Neugen). Pulsed field gel electrophoresis (PFGE) was performed with the parental and the antibiotic-resistant mutants following *Xba*I digestion. PFGE patterns of the antibiotic-resistant mutants were indistinguishable from those of the parental strains. The nalidixic acid- and rifampicin-resistant mutants were used instead of the parental strains in the experiments.

Inoculation

Bacterial strains, grown statically in TSB at 37 °C for 20 h, were harvested by centrifugation then washed once in phosphate

buffered saline (PBS) prior to appropriate dilution in PBS and use as individual inocula or combined as a 3-strain cocktail. Depending on the experiment, inocula containing 5 to 7 log₁₀ CFU/mL were randomly deposited on adaxial and abaxial sides of lettuce leaves in multiple 5 µL droplets. Inoculated lettuce leaves were left in a biological safety hood for 30 to 60 min to allow the droplets to evaporate and/or to adsorb to the leaf surfaces, followed by storage for 20 h at 4 °C in sealed plastic bags.

To assess the effect of washing and cutting sequences on the efficacy of chlorine washing, samples of 250 g trimmed Romaine lettuce leaves were inoculated with a 3-strain cocktail of *E. coli* O157:H7 strains (B6914/pGFP, ATCC 43888, and ATCC 700728) using quantitative surface spot inoculation. An inoculum of 300 µL of the cocktail, containing ca. 2×10^7 CFU/mL for each of the 3 strains, was randomly placed on the leaf surface as 5 µL droplets, resulting in an average ca. 2.4×10^4 CFU/g inoculation (calculated inoculation: 2.3, 1.6, and 2.6×10^4 CFU/g for the 3 strains, respectively, based on plating of inocula on CT-SMAC plates supplemented with corresponding antibiotics).

To evaluate the effect of washing and cutting sequences on the transference of bacterial pathogens during cutting and washing, "Lollo Rosa" red-leaf lettuce (25 g) samples were inoculated with *E. coli* O157:H7 strain CDC B6914/pGFP. Overnight culture in TSB was harvested and diluted in PBS as described above. An inoculum of 200 µL diluted cultures was randomly placed on the surfaces of 25 g red leaves as multiple 5 µL droplets. The calculated inoculation level was 3.5×10^3 CFU/g.

Lettuce cutting and washing

A produce slicer (North Star Engineered Products, Perrysburg, Ohio, U.S.A.) was used for cutting lettuce to 1/8" slices. Chlorinated water (70 ppm) was prepared by adding sodium hypochlorite in tap water, followed by adjusting the pH level to approximately 6.5 using 1.0 N hydrochloric acid (HCl). Each sample of whole or sliced lettuce leaves was washed with moderate manual agitation in chlorinated water at a 1:40 lettuce to water ratio for 30 or 60 s. Washed lettuce was de-watered in a manually actuated salad spinner (OXO Good Grips, Elmira, N.Y., U.S.A.) for 30 s before proceeding to next step or sampled.

For the experiments assessing the effect of washing and cutting sequences on the efficacy of chlorine washing for microbial reduction, inoculated Romaine lettuce leaves were cut to 1/8" slices and 3 25 g samples were taken for microbiological analysis to determine the background microbial flora and the recovery of the inoculated *E. coli* O157:H7 cells (Control). Two cutting-washing sequences were tested. In 1 treatment, inoculated Romaine lettuce leaves were cut as in the control, and washed in chlorinated water (70 ppm) for 1 min, followed by a 2nd wash in chlorinated water for 30 s (Cut-Wash). Three 25 g samples were taken for microbiological analyses after each washing. For the 2nd treatment, inoculated Romaine lettuce leaves were washed in chlorinated water (70 ppm) for 1 min and de-watered by centrifugation in a salad spinner, followed by cutting and a 2nd wash in chlorinated water for 30 s (Wash-Cut). Three 25 g samples were taken for microbiological analyses after cutting and after the 2nd wash. Each cutting-washing sequence was repeated 3 times.

To evaluate the effect of washing and cutting sequences on the transference of bacterial pathogens during cutting and washing, inoculated Lollo Rosa red lettuce leaves (25 g) were mixed with 250 g of freshly trimmed Green-Leaf lettuce leaves before subjecting them to treatments as outlined above. For the control, red- and green-lettuce leaf pieces were manually sorted after cutting. Four

4 g red lettuce subsamples (each representing 16% of originally inoculated red lettuce) and 20 g green lettuce subsamples (each representing 1.6% of noninoculated green lettuce) were taken for microbiological analyses. Cut red- and green-lettuce was similarly sorted and sampled after the 2nd wash for both Cut-Wash and Wash-Cut treatment sequences. All the treatments were repeated 3 times.

Water quality analyses

Water samples were taken before and after washing lettuce samples. A chlorine photometer (CP-15, HF Scientific Inc., Ft. Myers, Fla., U.S.A.) was used to measure free chlorine after appropriate dilutions for each water sample. A single aliquot from a free chlorine powder pop reagent was added to 5 mL of each diluted sample, vortexed to mix thoroughly and then 3 mL were transferred to a cuvette and measured against a distilled-water blank to obtain the free chlorine present in the sample as a function of absorbance. Total dissolved solids (TDS) were measured using a TDS/conductivity meter (Orion Research Inc., Beverly City, Mass., U.S.A.).

Bacteria enumeration

Each lettuce sample was mixed with 5 volumes of 0.2% buffered peptone water (BPW) or TSB in a filter stomacher bag and subjected to ultrasonic pulses (60 Hz) for 20 s in an ultrasound water bath (Alcar Industries, Belleville, N.J., U.S.A.), followed by stomaching for 2 min in a stomacher (Seward, Bohemia, N.Y., U.S.A.). Aliquots of the liquid phase were used for microbial enumeration using either direct plating or a microplate-based Most Probable Number (MPN) procedure. Background microbial flora on lettuce before and after washing was determined by plating appropriate dilutions of the stomacher liquid on Petrifilm aerobic plate count (APC) plates (3M Microbiology, St. Paul, Minn., U.S.A.) following manufacturer's instructions. Recovery of the inoculated *E. coli* O157:H7 cells were determined by spiral plating aliquots of the stomacher liquid on CT-SMAC plates supplemented with ampicillin (100 mg/L, Sigma, St. Louis, Mo., U.S.A.), nalidixic acid (50 mg/L, Sigma), or rifampicin (50 mg/L, Sigma). Characteristic colonies on the selective plates were confirmed as *E. coli* O157 by agglutination assay using DrySpot O157 (Oxoid, Cambridge, U.K.) following overnight incubation at 37 °C. A microplate-based 5-well (2.0 mL/well) 4-dilution MPN procedure was used to quantify the recovery of *E. coli* O157:H7 B6914/pGFP in a pathogen transfer experiment, in which lower level of inoculation was used. In this case, the samples (4 g) were sonicated and stomached in 20 mL of TSB containing 50 mg/L of ampicillin. Five 2 mL aliquots were serially diluted in TSB-ampicillin in a deep-well microplate and incubated overnight at 37 °C. Following the enrichment, 3 µL droplets of all the dilutions were arrayed on CT-SMAC plates supplemented with ampicillin (100 mg/L) using a multi-channel pipette. After 6 h incubation at 37 °C, the growth of *E. coli* O157:H7 B6-914/pGFP was confirmed by GFP fluorescence and the growth pattern was recorded for MPN calculation using a freeware MPN calculator available online (Curiale 2004). The remaining 10 mL of the stomacher liquid was enriched overnight and plated on CT-SMAC plates to determine the percentage of *E. coli* O157:H7 positive subsamples.

Statistic analyses

The experiment was conducted using a factorial design with washing sequences, and the number of washes as main factors. The experiment included 3 replications, with 3 samples per replication.

Data were analyzed as a 2-factor linear model using the PROC MIXED procedure (SAS Institute Inc., Cary, N.C., U.S.A.). Normality and variance homogeneity of the linear model were checked for the log transformed data. When effects were statistically significant, means comparisons were done with Sidak adjusted *P* values to maintain experiment-wise error ≤ 0.05 .

Results and Discussion

Effect of cutting and washing sequences on the efficacy of chlorine in lettuce microbial reduction

To assess the effect of washing and cutting sequences on the efficacy of chlorine washing, Romaine lettuce leaves were quantitatively inoculated with a 3-strain cocktail of *E. coli* O157:H7. Inoculated Romaine leaves were cut either prior to (Cut-Wash) or after (Wash-Cut) sanitizer washing in chlorinated water.

Both background microbial flora and inoculated *E. coli* O157:H7 strains were significantly reduced after washing in chlorinated water (Figure 1A). Compared to unwashed control (No Wash), washing freshly cut Romaine lettuce in chlorinated water for 60 s (Cut-Wash treatment) resulted in 1.1 log unit reduction (from 6.3 to 5.2 log CFU/g) in APC, and a 2nd wash in fresh chlorinated water for 30 s resulted in an additional 0.6 log unit reduction (to 4.6). In contrast, washing whole Romaine leaves in chlorinated water for 60 s followed by slicing (Wash-Cut treatment) resulted in 1.9 log unit reduction (from 6.3 to 4.4) in APC, and a 2nd wash resulted in an additional 0.4 log unit reduction (to 4.0). Taken together, altering the processing sequence by washing whole leaves in chlorinated water before cutting achieved 0.6 log unit higher reduction for the background flora than the traditional chlorine wash after cutting processing sequence when all other conditions were set equal.

Although variations were found on the recovery rates of different *E. coli* O157:H7 strains, all 3 showed similar patterns in response to the wash treatment (Figure 1B–1D). After lettuce was cut without washing treatment (No Wash), *E. coli* O157:H7 cells were recovered at 3.8, 3.4, and 3.6 log CFU/g for the 3 inoculated strains (CDC B6914/pGFP, ATCC 43888, and ATCC 700728, respectively). Washing in chlorinated water immediately following cutting (Cut-Wash treatment, 1st Wash) reduced *E. coli* O157:H7 counts by 1.3, 1.5, and 1.4 log units (to 2.5, 1.9, and 2.2 log CFU/g), respectively, for the 3 inoculated strains. However, a 2nd wash did not result in significant ($P > 0.05$) additional reduction for any of the strains tested (to 2.4, 1.8, and 2.2). On the other hand, washing lettuce in chlorinated water prior to cutting (Wash-Cut treatment, 1st Wash) significantly ($P < 0.001$) improved the *E. coli* O157:H7 reduction than that achieved by washing after cutting, although more variations among the different strains were observed. For strain CDC B6914/pGFP, a 2.0 log unit reduction (from 3.8 to 1.8) was observed. A 2nd wash in chlorinated water did not result in further reduction (Figure 1B). We were unable to recover strain ATCC 43888 by direct spiral plating method when inoculated leaves were cut after washing in chlorinated water. Considering that the calculated limit of detection by our experimental design was 0.5 log CFU/g, a decrease from the initial 3.4 log unit (No Wash control) represented more than a 2.9 log unit reduction for this strain (Figure 1C). For strain ATCC 700728, *E. coli* O157:H7 cells were recovered in 1 of the 3 repeats after the 1st wash and 2 of the 3 repeats after the 2nd wash, representing 2.9 and 2.0 log reduction, respectively (Figure 1D). The higher recovery of strain ATCC 700728 following the 2nd wash was puzzling. It could represent an artifact during the

experiment. Overall, chlorine washing prior to cutting resulted in 0.6 to 1.3 log unit higher reduction of *E. coli* O157:H7 than chlorine washing after cutting.

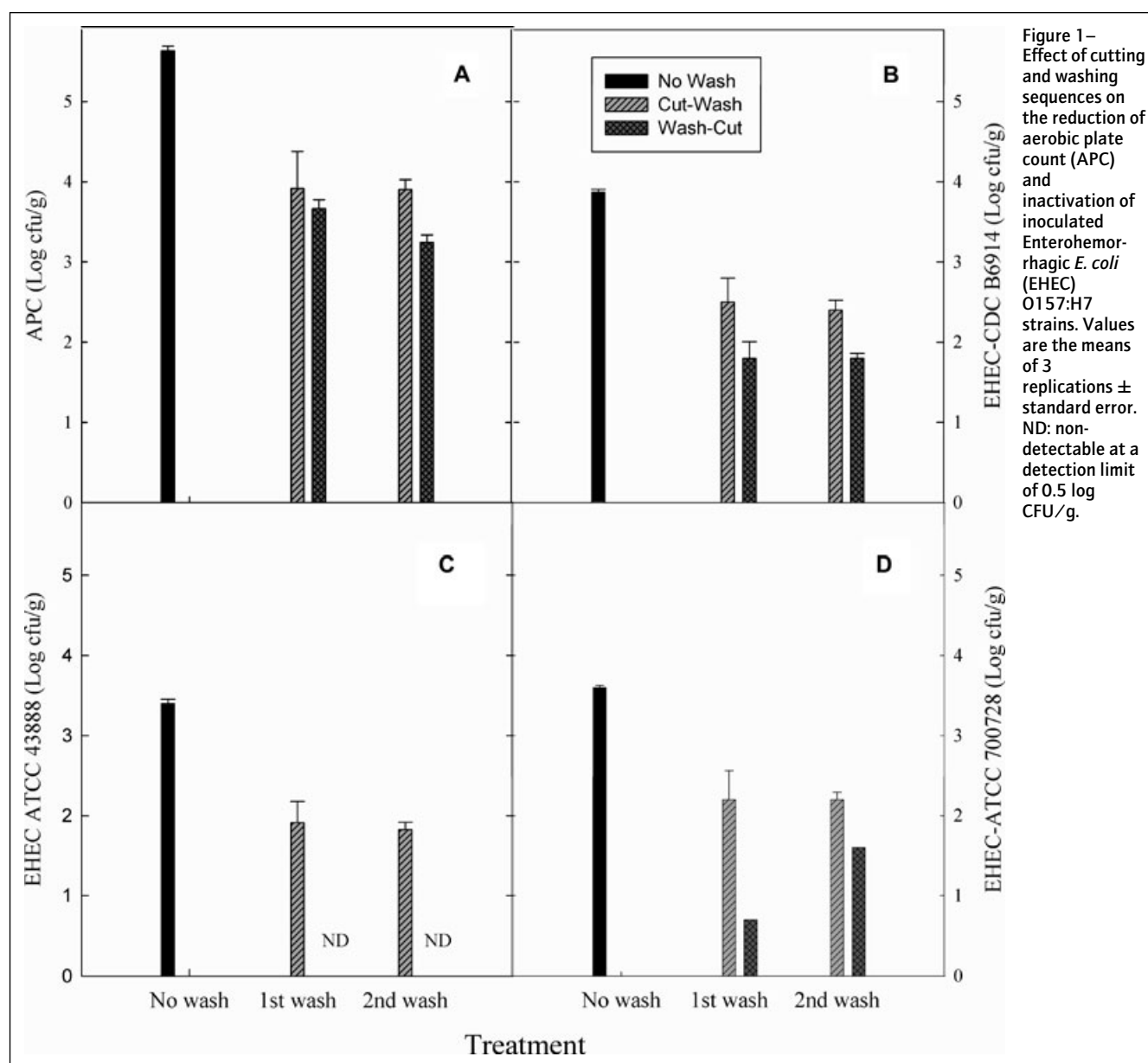
Effect of cutting and washing sequences on the quality of the washing water

The sequence of cutting and washing significantly affected the water quality in terms of free chlorine concentration and TDS (Figure 2). Free chlorine level in the wash solution dropped from 70 to 43 ppm when 250 g of cut lettuce was washed in 10 L of chlorinated water for 60 s, while TDS increased from 153 to 213 mg/L. A 2nd wash of the remaining cut lettuce (approximately 150 g; decreased mass was due to the removal of 75 g samples and other operational losses) in fresh chlorinated water did not significantly reduce the free chlorine level, and TDS only increased from 153 to 182 mg/L. No significant ($P > 0.05$) decrease of free chlorine was observed when the same amounts of lettuce leaves were washed prior to cutting, and there was only marginal increase

in TDS (from 153 to 157 mg/L). Furthermore, when previously washed lettuce leaves were cut and subjected to a 2nd wash, free chlorine level decreased from 70 to 59 ppm, and TDS increased from 153 to 192 mg/L. These data for previously washed lettuce suggest that the latex released from the cut surface of lettuce leaves has a major role in the increase in TDS and the decrease in free chlorine in the wash solution. The lack of significant changes in these parameters between 1st and 2nd washes for cut lettuce shows that 1 wash was sufficient for removing the majority of the latex.

Effect of cutting and washing sequence on the efficacy of chlorine for preventing pathogen cross-contamination

To evaluate the effect of washing and cutting sequences on the transference of bacterial pathogens during cutting and washing, Lollo Rosa red lettuce was quantitatively inoculated with *E. coli* O157:H7 strain CDC B6914/pGFP and mixed with non-inoculated Green-Leaf lettuce. The mixed lettuce leaves were cut prior to or after a sanitizing wash, followed by a 2nd



wash in chlorinated water. Red- and green-leaf slices were sorted and multiple subsamples were taken for microbiological analyses.

Significant *E. coli* O157:H7 transference from inoculated red lettuce to noninoculated green lettuce during cutting and possibly the ensuing sorting process was observed (Figure 3). For the control (No Wash), all of the red (12/12) and green (60/60) lettuce subsamples were positive for *E. coli* O157:H7, indicating that the cutting process is critically important for disseminating originally localized contamination. When lettuce leaves were washed in chlorinated water after cutting (Cut-Wash treatment), all of the red lettuce subsamples remained positive (12/12) after 2 consecutive chlorine washes while 95% of the green lettuce samples were positive for 2 of the 3 repeats and 100% of the green samples were positive for the other repeat (58/60). However, when inoculated and noninoculated lettuce leaves were mixed and subsequently washed together in chlorinated water prior to cutting (Wash-Cut treatment), *E. coli* O157:H7 transference and survival were greatly reduced. Among the 3 repeats, *E. coli* O157:H7 was recovered

in 1 red lettuce subsample (1/12) and 2 green lettuce subsamples (2/60). This demonstrated that chlorine washing when performed at the whole-leaf stage was more effective at preventing pathogen transference from initially localized contamination spots to other leaves.

Figure 4 shows the distribution of the cell counts of each of the subsamples. When mixed lettuce leaves were cut without chlorine washing treatment (No Wash controls), the *E. coli* O157:H7 cell counts for the inoculated samples (red lettuce) ranged from 1.9 to ≥ 3.6 log CFU/g, with an average of ≥ 3.2 log CFU/g. Since each subsample represented 16% of the red lettuce mass in the whole sample, this indicated that a total of ≥ 4.6 log CFU remained on 25 g inoculated red lettuce. The average cell count estimate for noninoculated green lettuce (Each subsample represented 1.6% of the green-leaf lettuce in the whole sample) was 1.9 log CFU/g, ranging from 0.8 to 2.6 log CFU/g, which represents a total of 4.3 log CFU in the 250 g noninoculated green lettuce. Therefore, approximately 30% of the recovered *E. coli* O157:H7 were transferred from red to green lettuce during the cutting and

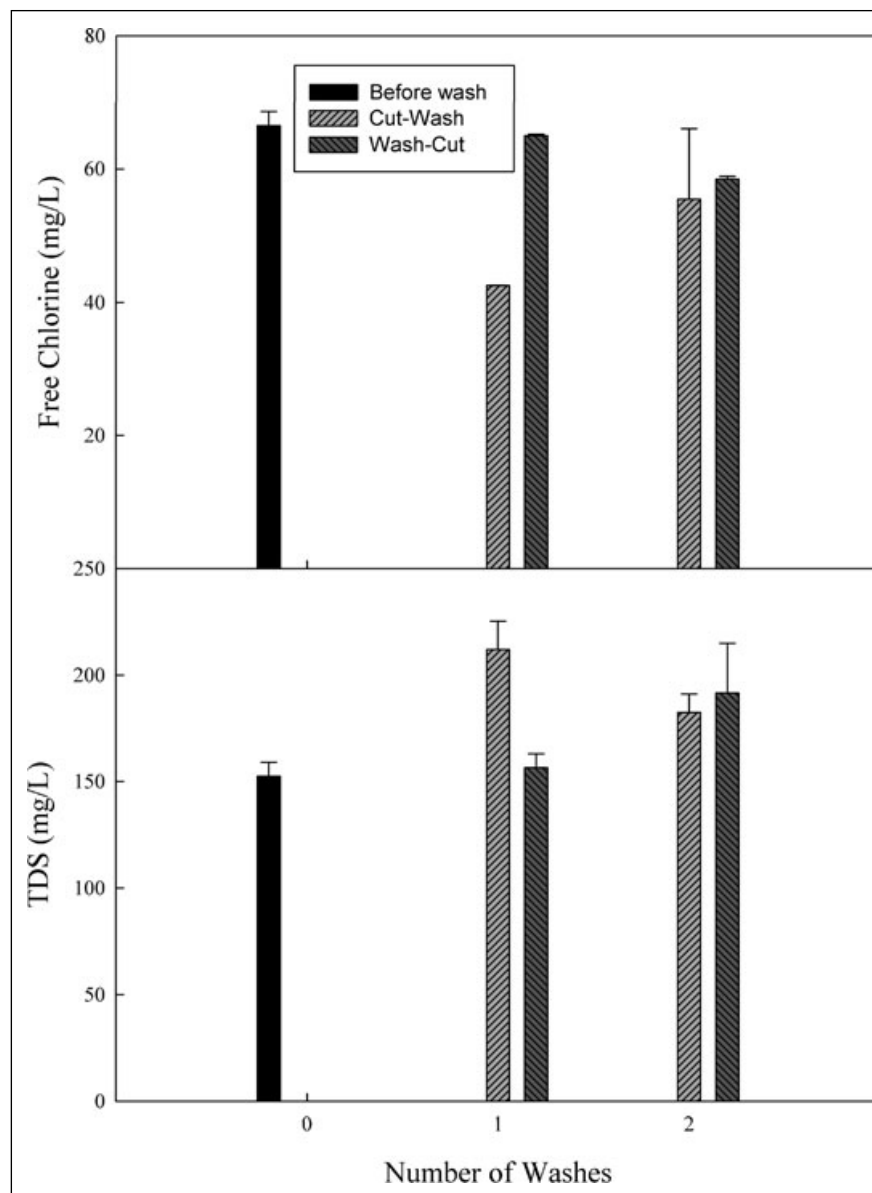


Figure 2—Effect of cutting and washing sequences on the changes of water quality during fresh-cut produce washing process. Values are the means of 3 replications \pm standard error.

sorting process. The observed transfer may be higher than what might be encountered in reality for 2 reasons: (1) difficulty in eliminating very small pieces of red-leaf lettuce attached to green-leaf pieces during sorting; (2) freshly transferred cells might be more readily recoverable than those firmly attached to the red lettuce surface. Indeed, there were 4 green-leaf subsamples with abnormally high counts of *E. coli* O157:H7, which could be resulted from red piece contamination.

For subsamples from the Cut-Wash treatment groups, the inoculated red lettuce had an average *E. coli* O157:H7 cell count of 1.6 log CFU/g, ranging from 0.9 to 2.1. For the noninoculated green lettuce, 16 of the 58 positive subsamples had *E. coli* O157:H7 cell counts below the enumeration limit of -0.3 log CFU/g by the MPN procedure used in this experiment. *E. coli* O157:H7 cell counts averaged ≤ 0.6 log CFU/g, ranging from ≤ -0.3 to 1.4 log CFU/g for all the positive green lettuce subsamples. This corresponds to approximately 44% of the recovered *E. coli* O157:H7 being transferred from red lettuce leaves (3.0 log CFU remaining) to green lettuce leaves (≤ 2.9 log CFU). Assuming equal amounts of *E. coli* transferred from red to green lettuce leaves during cutting as for the unwashed controls, this result indicates that about 5% of the transferred *E. coli* O157:H7 cells survived 2 chlorine washes (0.6 compared with 1.9 log CFU/g for Cut-Wash treatment compared with No Wash control group, 1.3 log unit reduction), while approximately 3% of the inoculums remaining on red lettuce survived the chlorine washes (1.6 compared with 3.2 log CFU/g, 1.6 log unit reduction).

Chlorine washing prior to cutting (Wash-Cut treatment) not only more effectively prevented *E. coli* O157:H7 transference from inoculated red lettuce to noninoculated green lettuce, it also resulted in more efficient pathogen inactivation on the contaminated lettuce leaves. Among the 3 repeats, *E. coli* O157:H7 was recovered in 1 red lettuce subsample, with MPN value of 2 CFU/g (0.3 log CFU/g), and 2 green lettuce subsamples, with MPN value below the enumeration limit for the procedure applied in this experiment (≤ -0.3 log CFU/g). This would correspond to more than 2.9 and 2.2 log unit reductions for *E. coli* O157:H7 on red and green lettuce leaves, respectively.

In many fresh-cut leafy green processing plants, chlorinated water washing is often the only antimicrobial step designed to remove potential contamination of bacterial pathogens (Garg and others 1990). The antimicrobial effectiveness of chlorine and other oxidant sanitizers is significantly affected by the organic loads in the washing solution (Gonzalez and others 2004; Luo 2007; Zhang and others 2009). When freshly cut lettuce is discharged into the washing system, the sudden surge of organic matter in the washing solution resulting from the introduction of freshly released vegetable latex and the lettuce tissue can lead to temporal depletion of free chlorine, creating an opportunity for bacterial pathogen survival and spread among lettuce tissues within the batch wash. Processing plants closely monitor the free chlorine levels in wash solutions and periodically replenish them with fresh chlorine to meet a standard operational concentration. However, maintaining free chlorine level is a technically challenging process when there are frequent organic load surges. Keeping very high chlorine levels adequate to handle a high organic load surge is not only costly, but also creates an increased health hazard for employees from elevated chlorine off-gassing in confined work areas. It has also been demonstrated that chlorine by-products can form potentially carcinogenic compounds (Connell 1996; Parish and others 2003), which need to be avoided on food products.

The cutting process generates large amounts of freshly wounded surfaces on the lettuce leaf tissues. It has been demonstrated that bacterial pathogens such as *E. coli* O157:H7 preferentially attach to these cut edges and wounded tissues (Takeuchi and Frank 2000; Li and others 2001). Once *E. coli* O157:H7 cells attach to the wounded surfaces, they become very difficult to remove by sanitizer washing (Beuchat 2004). Moreover, *E. coli* O157:H7 cells attached to wounded surfaces could infiltrate into the tissue and become protected from antimicrobial agents or processes (Li and others 2008). Wounded lettuce tissue can provide sufficient nutrition for continued multiplication of bacterial pathogens such as *E. coli* O157:H7 (McEvoy and others 2009).

Problems associated with contamination of wounded lettuce tissues can be, at least, partially avoided by washing lettuce leaves in chlorinated water prior to cutting. Without the large amounts of

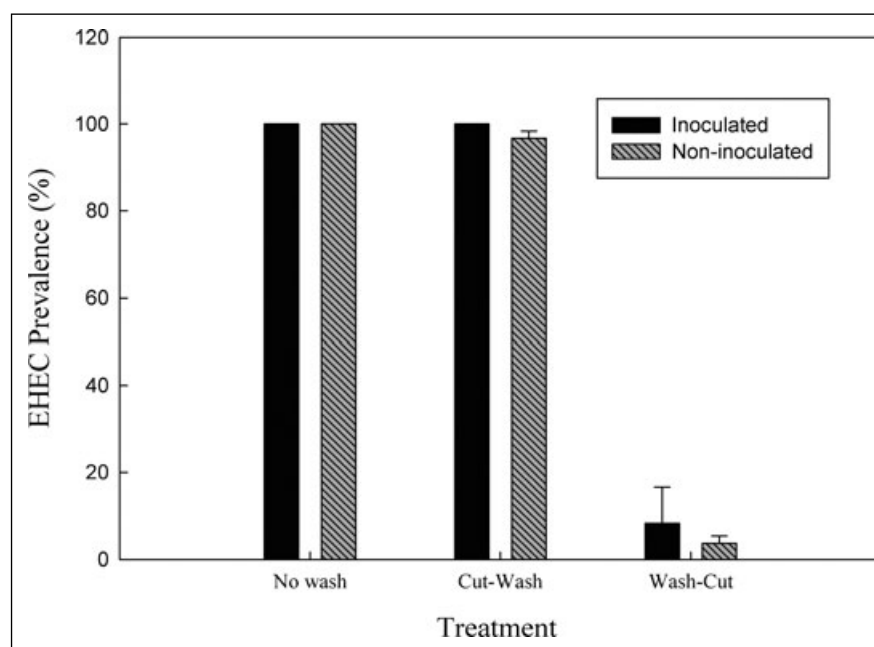


Figure 3—Effect of cutting and washing sequence on the transference of *E. coli* O157:H7 cells from inoculated (red lettuce) to noninoculated (green lettuce) products. Transference was measured using the prevalence (%) of positive subsamples. Values are the means of 3 replications \pm standard error.

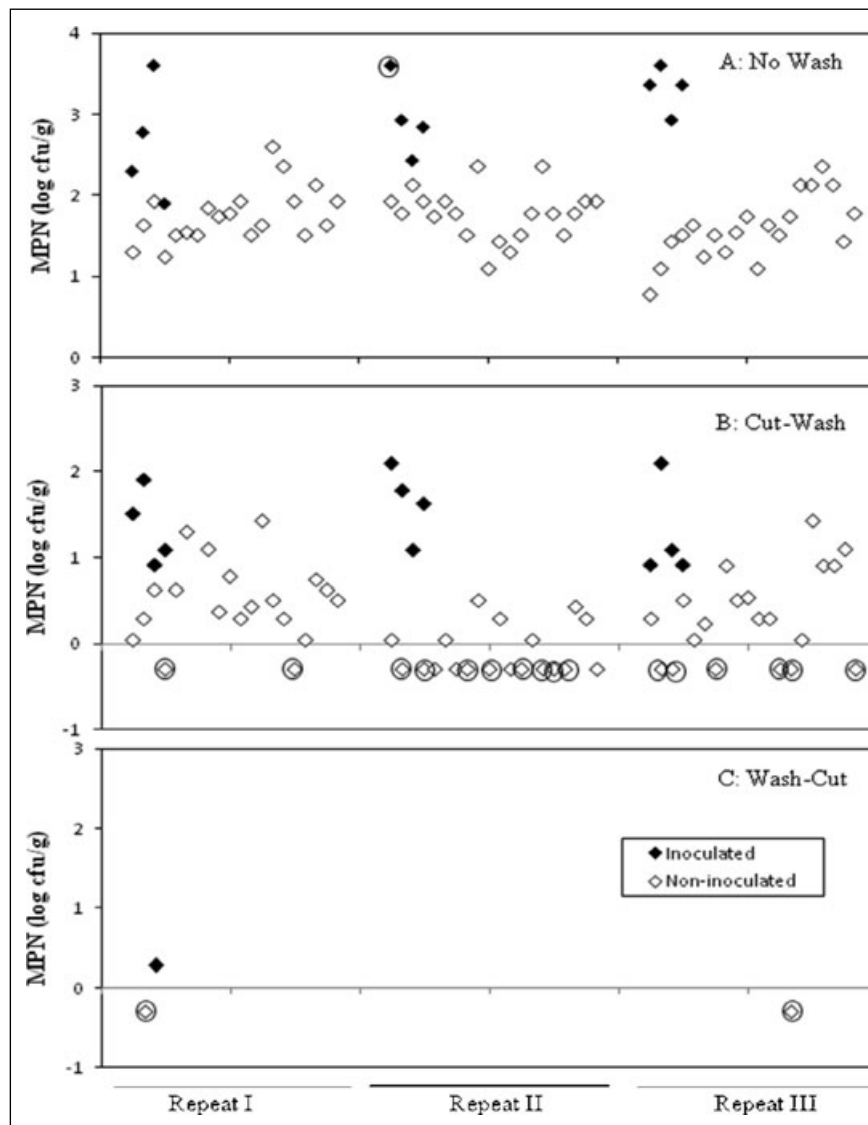


Figure 4—Quantification of *E. coli* O157:H7 transference affected by the cutting and washing sequences. Each diamond represents the MPN value of one subsample. The range for MPN enumeration was -0.3 to 3.6 log CFU/g. Solid diamond inside a circle indicates MPN value over the limit of the MPN enumeration procedure in this experiment. An unfilled diamond inside a circle indicates MPN value below the limit of enumeration.

organic fluids discharged into the washing systems from the cut leaves, an adequate sanitizing level of free chlorine can be more easily maintained and the washing efficacy will be improved. Most of the pathogen cells will be inactivated before the large amounts of wounded surfaces being generated by cutting. The data presented here strongly support this assertion. Depending on the type of produce, chlorine wash prior to cutting can improve microbial reduction by 0.7 to 2 logs over postcutting washes. It also significantly reduces the potential for pathogen cross-contamination during the ensuing cutting process.

Fresh-cut leafy green processing plants are traditionally configured for chlorine washing following cutting. This configuration was established before leafy greens were recognized as a significant vehicle of food-borne bacterial pathogens. Traditionally, the slicing equipment required that lettuce leaves be uniformly oriented for cutting, which made whole-leaf washing before cutting impractical. Technical advances on fresh-cut processing equipment, especially its capacity to handle field cored/trimmed Romaine lettuce have made the practice of whole-leaf washing possible. Therefore, instituting a sanitizing wash prior to lettuce cutting

can be a practical and cost-effective solution for improving chlorine washing efficacy and minimizing potential bacterial pathogen cross-contamination.

Conclusion

Fresh-cut leafy green processors traditionally apply a sanitizer wash following cutting as the main antimicrobial intervention. Sanitizer washing at this stage is not optimally effective on pathogen reduction, because leaf exudates neutralize sanitizers commonly employed for fresh-cut processing. The current study demonstrated that a whole-leaf wash approach applied prior to cutting significantly improved the efficacy of sanitizer washing and also significantly reduced the potential of cross-contamination by bacterial pathogens during the cutting and other processing steps.

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References

- Adams MR, Hartley AD, Cox LJ. 1989. Factors affecting the efficiency of washing procedures used in the production of prepared salads. *Food Microbiol* 6:69–77.
- Beuchat L. 2004. Difficulties in eliminating human pathogenic microorganisms on raw fruits and vegetables. *Acta Hort* 642:151–60.
- CA-LGMA. 2007. Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens. Available from: <http://www.caleafygreens.ca.gov/members/documents/LGMAAacceptedGAPs06.13.08.pdf>.
- Connell G. 1996. The chlorination/chloramination handbook. Denver, CO: American Water Works Association.
- Curiale M. 2004. MPN calculator (VB6 version) for food, feed, and water microbiologist. Available from: www.i2workout.com/mcuriale/mpn/index.html.
- FDA. 2006. Commodity specific food safety guidelines for the lettuce and leafy greens supply chain. Available from: <http://www.fda.gov/downloads/Food/FoodSafety/Product-specificInformation/FruitsVegetablesJuices/GuidanceComplianceRegulatoryInformation/UCM169008.pdf>.
- Franz E, van Bruggen AH. 2008. Ecology of *E. coli* O157:H7 and *Salmonella enterica* in the primary vegetable production chain. *Crit Rev Microbiol* 34(3–4):143–61.
- Garg N, Churey JJ, Splittstoesser DF. 1990. Effect of processing conditions on the microflora of fresh-cut vegetables. *J Food Prot* 53:701–3.
- Gil MI, Selma MV, Lopez-Galvez F, Allende A. 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. *Int J Food Microbiol* 134(1–2):37–45.
- Gonzalez RJ, Luo Y, Ruiz-Cruz S, McEvoy JL. 2004. Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *J Food Prot* 67(11):2375–80.
- Herman K, Ayers T, Lynch M. 2008. Foodborne disease outbreaks associated with leafy greens, 1973–2006. Available from: http://www.cdc.gov/ncidod/EID/announcements/iceid_2008.htm.
- Islam M, Doyle MP, Phatak SC, Millner P, Jiang X. 2004. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *J Food Prot* 67(7):1365–70.
- Jay MT, Cooley M, Carychao D, Wiscomb GW, Sweitzer RA, Crawford-Miksza L, Farrar JA, Lau DK, O'Connell J, Millington A, Asmundson RV, Atwill ER, Mandrell RE. 2007. *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, Central California Coast. *Emerg Infect Dis* 13:1908–21.
- Kim JK, Harrison MA. 2008. Transfer of *Escherichia coli* O157:H7 to romaine lettuce due to contact water from melting ice. *J Food Prot* 71(2):252–6.
- Li Y, Brackett RE, Chen J, Beuchat LR. 2001. Survival and growth of *Escherichia coli* O157:H7 inoculated onto cut lettuce before or after heating in chlorinated water, followed by storage at 5 or 15 degrees C. *J Food Prot* 64(3):305–9.
- Li H, Tajkarimi M, Osburn BI. 2008. Impact of vacuum cooling on *Escherichia coli* O157:H7 infiltration into lettuce tissue. *Appl Environ Microbiol* 74(10):3138–42.
- Luo Y. 2007. Wash operation affect water quality and packaged fresh-cut romaine lettuce quality and microbial growth. *HortScience* 42:1413–9.
- Lynch MF, Tauxe RV, Hedberg CW. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiol Infect* 137(3):307–15.
- McEvoy JL, Luo Y, Conway W, Zhou B, Feng H. 2009. Potential of *Escherichia coli* O157:H7 to grow on field-cored lettuce as impacted by postharvest storage time and temperature. *Int J Food Microbiol* 128(3):506–9.
- Parish M, Beuchat LR, Suslow TV, Harris LJ, Garrett EH, Farber JN, Busta FF. 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce, chapter V, in analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. *Compr Rev Food Sci Food Saf* 2:161–73.
- Solomon EB, Pang HJ, Matthews KR. 2003. Persistence of *Escherichia coli* O157:H7 on lettuce plants following spray irrigation with contaminated water. *J Food Prot* 66(12):2198–202.
- Takeuchi K, Frank JF. 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *J Food Prot* 63(4):434–40.
- Taormina PJ, Beuchat LR, Erickson MC, Ma L, Zhang G, Doyle MP. 2009. Transfer of *Escherichia coli* O157:H7 to iceberg lettuce via simulated field coring. *J Food Prot* 72(3):465–72.
- Wachtel MR, Whitehand LC, Mandrell RE. 2002. Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. *J Food Prot* 65(1):18–25.
- Zhang G, Ma L, Phelan VH, Doyle MP. 2009. Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157:H7. *J Food Prot* 72(7):1392–7.